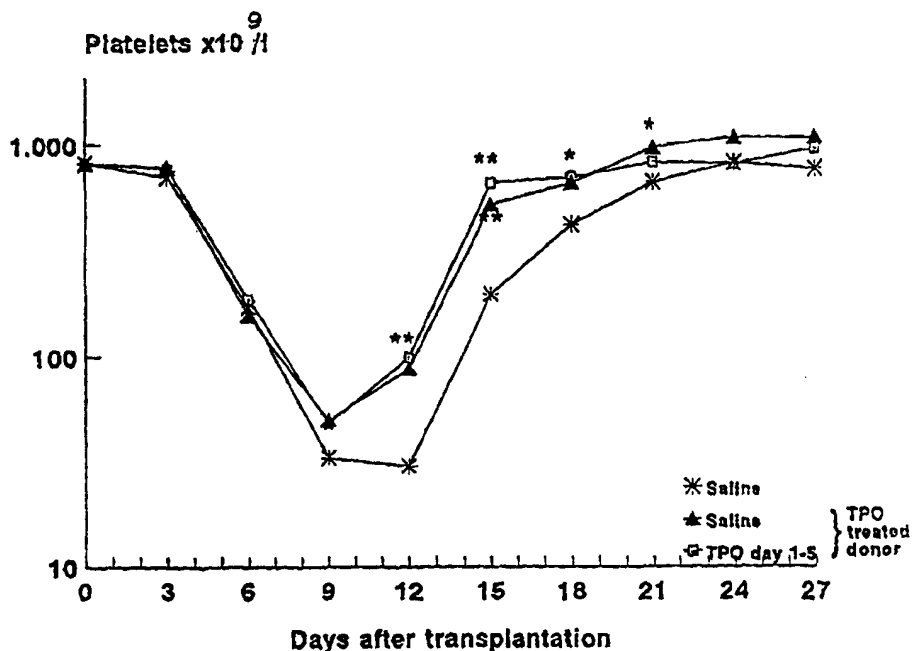




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(54) Title: METHODS FOR INCREASING HEMATOPOIETIC CELLS



(57) Abstract

Methods for increasing hematopoietic cells, including platelets and erythrocytes, in patients receiving bone marrow or peripheral blood stem cell transplants are disclosed. The methods comprise administering to a donor an amount of thrombopoietin sufficient to stimulate proliferation of cells of the myeloid lineage, collecting cells from the donor, and administering the collected cells to a recipient patient. The recipient patient may be treated with additional thrombopoietin. The methods are useful within allogeneic and autologous transplantation procedures.

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Description

5 METHODS FOR INCREASING HEMATOPOIETIC CELLS

Background of the Invention

Hematopoiesis is the process by which blood cells develop and differentiate from pluripotent stem
10 cells in the bone marrow. This process involves a complex
interplay of polypeptide growth factors (cytokines) acting
via membrane-bound receptors on their target cells.
Cytokine action results in cellular proliferation and
differentiation, with a response to a particular cytokine
15 often being lineage-specific and/or stage-specific.
Development of a single cell type, such as a platelet,
from a stem cell may require the coordinated action of a
plurality of cytokines acting in the proper sequence.

It was hypothesized for many years that the
20 production of platelets may be regulated by specific
humoral factors. Early experiments had shown that plasma
or urine of thrombocytopenic animals contains an activity
that promotes megakaryocytic colony formation and
increases the size of marrow megakaryocytes. This
25 activity is referred to in the literature as
"thrombopoietin" (recently reviewed by McDonald, Exp.
Hematol. 16:201-205, 1988 and McDonald, Am. J. Ped.
Hematol. Oncol. 14:8-21, 1992). The low concentration of
this activity and the lack of suitable bioassays long
30 hampered the purification and characterization of the
protein. Thrombopoietin has now been produced using
genetically engineered cultured cells. See, de Sauvage et
al., Nature 369:533-538, 1994; Lok et al., Nature
369:565-568, 1994; Kaushansky et al., Nature 369:568-571,
35 1994; and Bartley et al., Cell 77:1117-1124, 1994.

Thrombopoietin has been shown to increase
platelet numbers in normal (Lok et al., *ibid.*) and

thrombocytopenic (Sprugel et al., Blood 84 (10 Suppl. 1):242a, 1994) animals, and to stimulate production of erythrocytes (Kaushansky et al., J. Clin. Invest., in press). In vitro, TPO enhances survival and proliferation
5 of CD34⁺ cells destined to become megakaryocytes (Papayannopoulou et al., Blood 84 (10 Suppl. 1):324a, 1994).

Although the cloning and characterization of TPO now permits investigation of its clinical use in
10 stimulating thrombopoiesis, thrombocytopenia and anemia remain as significant clinical problems, such as in connection with chemotherapy and radiation therapy of cancer patients. There remains a particular need for methods of stimulating platelet production in patients
15 receiving bone marrow transplants and peripheral blood stem cell transplants, including autologous transplants. There also remains a need for stimulating erythrocyte production. The present invention provides therapeutic methods that address these needs, and provides other,
20 related advantages.

Summary of the Invention

The present invention provides methods for increasing hematopoietic cells in a recipient patient in
25 need of such increase. The methods comprise the steps of (a) administering to a donor an amount of thrombopoietin (TPO) sufficient to stimulate proliferation of cells of the myeloid lineage in the donor; (b) collecting cells from the donor, wherein the cells are bone marrow cells or
30 peripheral blood stem cells; and (c) administering the bone marrow cells or peripheral blood stem cells to a recipient patient. The donor and recipient may be different individuals or the same individual. Within one embodiment of the invention, the recipient patient has
35 been treated with chemotherapy or radiation therapy. Within another embodiment, after or concurrently with administering the bone marrow cells or peripheral blood

stem cells, an amount of TPO sufficient to enhance platelet recovery or erythrocyte recovery is administered to the recipient patient.

Within another aspect, the present invention provides methods of preparing cells for transplantation comprising administering to a donor an amount of TPO sufficient to stimulate proliferation of cells of the myeloid lineage in the donor, and collecting cells from the donor, wherein the cells are bone marrow cells or peripheral blood stem cells.

Within a third aspect, the present invention provides a method of stimulating platelet recovery or erythrocyte recovery in a patient receiving chemotherapy or radiation therapy comprising (a) administering to the patient an amount of TPO sufficient to stimulate proliferation of cells of the myeloid lineage in the patient; (b) collecting bone marrow cells or peripheral blood stem cells from the patient prior to chemotherapy or radiation therapy; and (c) returning the collected cells to the patient subsequent to chemotherapy or radiation therapy. Within one embodiment this method further comprises administering to the patient, after or concurrently with returning the collected cells, an amount of TPO sufficient to enhance platelet recovery or erythrocyte recovery.

These and other aspects of the invention will become evident upon reference to the following detailed description and the attached drawings.

Brief Description of the Drawings

Fig. 1 illustrates the effect of transplantation of bone marrow cells from TPO- or vehicle-treated donor mice on platelet counts in recipient animals. In one experiment recipients of TPO-treated marrow were also treated with TPO (20 kU/day i.p.). Data are presented as means of 10-20 mice in two experiments. *, $p < 0.05$; **, $p < 0.01$.

Fig. 2 illustrates the effect of transplantation of bone marrow cells from TPO- or vehicle-treated donor mice on erythrocyte counts in recipient animals. Data are expressed as mean of 20 mice in two experiments. *,
5 p<0.05; **, p<0.005.

Fig. 3 illustrates platelet recovery in mice receiving marrow transplants from TPO- or vehicle-treated donors, with or without post-transplant TPO treatment.

10 Detailed Description of the Invention

The term "stem cell" is used herein to denote pluripotent hematopoietic stem cells and myeloid progenitor cells.

The term "transplantation" is used herein to
15 denote the process of removing cells from a donor and subsequently administering the cells to a recipient. The term encompasses both allogeneic transplantation, wherein the donor and recipient are different individuals of the same species; and autologous transplantation, wherein the
20 donor and recipient are the same individual.

The term "increasing hematopoietic cells" is used herein to denote the restoration or enhanced recovery of hematopoietic cell levels following their ablation, such as ablation resulting from disease or therapeutic
25 intervention.

The term "thrombopoietin" encompasses proteins characterized by their ability to specifically bind to MPL receptor from the same species and to stimulate platelet production *in vivo*. In normal test animals, TPO is able
30 to increase platelet levels by 100% or more within 10 days after beginning daily administration. A representative human TPO cDNA sequence is shown in SEQ ID NO: 1, and the corresponding amino acid sequence is shown in SEQ ID NO: 2. Analytical and experimental evidence indicates that
35 the mature protein begins at residue Ser-22. Those skilled in the art will recognize that the illustrated sequences correspond to a single allele of the human TPO

gene, and that allelic variation is expected to exist. Allelic variants include those containing silent mutations and those in which mutations result in amino acid sequence changes. It will also be evident that one skilled in the art could create additional variants, such as by engineering sites that would facilitate manipulation of the nucleotide sequence using alternative codons, by substitution of codons to produce conservative changes in amino acid sequence, etc. The use of allelic and engineered variant TPOs is contemplated by the present invention. In addition, amino-terminal TPO polypeptides of about 150 amino acids or more in length are known to be active (de Sauvage et al., *ibid.*; Bartley et al., *ibid.*; co-pending, commonly assigned U.S. Patent application Serial No. 08/346,999), and the use of such truncated forms of TPO is within the scope of the present invention. Thrombopoietins from non-human species have been disclosed in the scientific literature (Lok et al., *ibid.*; de Sauvage et al., *ibid.*; Bartley et al., *ibid.*).

The present invention provides methods for increasing hematopoietic cells in patients, particularly patients undergoing radiation therapy and/or chemotherapy, such as in the treatment of cancer. Such therapies kill dividing progenitor cells in the marrow and peripheral blood, limiting therapy and often requiring transfusions to restore circulating levels of platelets and other blood cells. Of particular interest are those patients receiving bone marrow and/or peripheral blood stem cell transplants following radiation therapy and patients suffering from congenital metabolic defects necessitating bone marrow transplant. Among these indications are bone marrow transplants associated with treatment of breast cancer, leukemia, lymphoma, multiple myeloma and congenital defects such as severe combined immune deficiency, thalassemia, and sickle cell anemia. Peripheral blood stem cell transplantation may be

preferred in conditions where a risk of tumor cells in the blood is not present.

Methods for carrying out bone marrow and peripheral blood stem cell transplants are known in the art. For a review, see Snyder et al., "Transfusion Medicine" in Benz and McArthur, eds., Hematology 1994, American Society of Hematology, 96-106, 1994. Peripheral blood stem cells are collected by leukapheresis according to accepted clinical procedures. Hematopoietic progenitor cells can be selected on the basis of cell surface markers (e.g. CD34), allowing for enrichment of the desired cells and depletion of contaminating tumor cells. The collected cells are stored frozen in a suitable cryoprotectant (e.g. dimethyl sulfoxide, hydroxyethyl starch) until needed. Marrow cells are collected from donors by bone puncture under anesthesia. To reduce the volume, the collected marrow is usually processed to separate plasma from the cellular components. Removal of plasma can also eliminate red cell incompatibilities in allogeneic transplantation. The cell fraction can be enriched for mononuclear cells using density gradient techniques or automated separation methods and depleted of T cells using various cytotoxic agents. Collected marrow cells are cryopreserved according to established procedures that include controlled-rate freezing and the use of cryoprotectants. Stem cells are thawed in a warm water bath immediately prior to use to minimize loss associated with thawing. In the case of allogeneic transplants, donors and recipients are tissue matched to minimize the risk of graft-versus-host disease.

An increase in hematopoietic cells results from transplantation into a recipient patient of stem cells, particularly cells of the myeloid lineage, including CD34⁺ stem cells and cells derived from CD34⁺ stem cells. Of particular interest are cells in the megakaryocyte and erythrocyte lineages, which reconstitute the recipient's platelet and erythrocyte populations, respectively.

Within the present invention, a donor is treated, prior to donation of marrow or peripheral blood cells, with TPO in an amount sufficient to stimulate proliferation of cells of the myeloid lineage. Such an amount will generally be in the range of 0.5 lg/kg/day to 40 lg/kg/day, preferably 1 lg/kg/day to 20 lg/kg/day. Treatment of the donor will be carried out for a period of from one to several days, preferably about 2-5 days, during a period of from 3 days to 2 weeks prior to harvesting of bone marrow or peripheral blood stem cells. It is preferred to treat the donor during a period of five to ten days prior to harvesting of cells. The increase in CD34⁺ stem cells and other cells of the myeloid lineage in the donor will be manifested by improved recovery of platelet and/or erythrocyte levels in the transplant recipient.

Within one embodiment of the invention, the recipient is treated with TPO after transplantation to further enhance platelet recovery. It has been found that post-transplantation treatment with TPO improves survival of lethally-irradiated test animals given bone marrow from TPO-treated donors. "An amount of thrombopoietin sufficient to enhance platelet recovery" is that amount that produces a statistically significant reduction in time for recovery of normal platelet levels or a statistically significant increase in platelet count as compared to untreated patients. Doses of TPO used in post-transplantation treatment will generally be in the range of 0.5 lg/kg/day to 40 lg/kg/day administered for from about 3 to about 20 days. In general, patients receiving bone marrow transplants will require longer post-transplantation treatment than those receiving peripheral blood stem cell transplants.

For use within the present invention, TPO can be prepared using genetically engineered, cultured cells according to methods generally known in the art. To summarize these methods, a DNA molecule encoding TPO is

joined to other DNA sequences which provide for its maintenance and transcription in a host cell. The resulting expression vector is inserted into the host cell, and the resulting "transformed" or "transfected" cells are cultured in a suitable nutrient medium. Baby hamster kidney (BHK) cells are a preferred host. It is preferred to engineer the cells to secrete the TPO into the medium, although TPO can be recovered from cell lysates and processed *in vitro* to yield active protein.

See, in general, de Sauvage et al., *ibid.*; Lok et al., *ibid.*; Kaushansky et al., Nature 369:568-571, 1994; Wendling et al., Nature 369:571-574, 1994; Bartley et al., *ibid.*; and co-pending, commonly assigned U.S. Patent Applications Serial No. 08/366,859 and Serial No. 08/347,029, which are incorporated herein by reference in their entirety.

TPO may be purified from cell-conditioned culture media by a combination of chromatographic and other techniques, including direct capture on a dye-ligand affinity matrix and ion-exchange chromatography. Contaminating proteins may be removed by adsorption to hydroxyapatite.

For pharmaceutical use, TPO is formulated for parenteral, particularly intravenous or subcutaneous, delivery according to conventional methods. Intravenous administration will be by bolus injection or infusion over a typical period of one to several hours. In general, pharmaceutical formulations will include a hematopoietic protein in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents (e.g. phosphate buffer), albumin or a non-ionic detergent to prevent protein loss on vial surfaces, etc.

In addition, TPO may be combined with other cytokines, particularly early-acting cytokines such as stem cell factor, IL-3, IL-6, IL-11 or GM-CSF. When utilizing such

a combination therapy, the cytokines may be combined in a single formulation or may be administered in separate formulations. Methods of formulation are well known in the art and are disclosed, for example, in Remington's
5 Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., Easton PA, 1990, which is incorporated herein by reference.

The invention is further illustrated by the following, non-limiting examples.

10

Examples

Example 1

Mouse thrombopoietin was prepared using transfected baby hamster kidney cells (BHK 570 cells, ATCC
15 CRL 10314). Serum-free medium contained 145 kU/ml of TPO activity, wherein 10 units are defined as the amount of TPO giving half-maximal stimulation in a mitogenesis (³H-thymidine incorporation) assay using BaF3 cells transfected with an expression vector encoding the human
20 MPL receptor (Vigon et al., Proc. Natl. Acad. Sci. USA 89:5640-5644, 1992) as target cells. BaF3 is an interleukin-3 dependent pre-lymphoid cell line derived from murine bone marrow (Palacios and Steinmetz, Cell 41:
727-734, 1985; Mathey-Prevot et al., Mol. Cell. Biol. 6:
25 4133-4135, 1986). Cells were exposed to test samples in the presence of ³H-thymidine. The amount of ³H-thymidine incorporated into cellular DNA was quantitated by comparison to a standard curve of human TPO. Mouse TPO
30 samples were effective in colony forming assays in a range of approximately 100-400 U/ml. In vivo activities were seen in the range of 20-40 kU/day in mice. For in vivo experiments, TPO was diluted to the desired concentration in endotoxin-free phosphate-buffered saline (PBS) and administered as intraperitoneal or subcutaneous
35 injections.

Female Balb-C mice (age range 8-12 weeks) were obtained from Broekman B.V. (Somerens, The Netherlands) and

fed commercially available rodent chow and provided with acidified water *ad libitum*. Transplant recipients were maintained in a pathogen-free environment and provided with water containing ciprofloxacin at a concentration of 1 mg/ml, polymyxine-B at 70 lg/ml, and saccharose at 2 g/100 ml.

Recipient mice were placed in a polymethylmetaacetate box and lethally (8.5 Gy) irradiated using a Philips SL 75-5/6 mV linear accelerator (Philips Medical Systems, Best, The Netherlands). Irradiation was divided in two parts in posterior-anterior and anterior-posterior position, at a dose rate of 4 Gy/minute. The mice were transplanted with 10^5 bone marrow cells from steady-state donor mice. Transplantation was carried out within four hours of marrow harvesting. Groups of 5 recipient mice were treated with TPO at a dose of 20 kU/day intraperitoneally (i.p.) on days 1-5, 3-8 or 3-12 after transplantation. Control animals were transplanted with an equal amount of marrow cells and given saline at similar time intervals after transplantation. In comparison with saline-treated control recipients, TPO administration did not result in accelerated platelet reconstitution. A dose of 30 kU/day administered subcutaneously (s.c.) on days 1-14 was also ineffective in accelerating platelet recovery. No effects were seen on reconstitution of white blood cells or red blood cells.

In a second set of experiments, donor mice were treated with TPO for five consecutive days at a dose of 20 kU/day i.p. per mouse. At day 5 the mice were sacrificed, and blood, bone marrow and spleens were harvested. White blood cells, red blood cells and platelets were counted on a Sysmex 800 counter (TOA Medical Electronics Company, Kobe, Japan). TPO treatment induced a 2.5-fold increase in the numbers of platelets, but had no effect on the numbers of white blood cells or red blood cells.

Progenitor cell levels were also determined in the TPO-treated donor mice. Bone marrow cells were

harvested by flushing femurs under sterile conditions with RPMI 1640 containing 500 lg/ml penicillin, 250 lg/ml streptomycin, and 2% fetal bovine serum (FBS) (GIBCO BRL, Gaithersburg, MD). Single-cell suspensions of the spleens
5 were prepared by mashing the organs and washing once with RPMI 1640 containing 2% FBS. To determine colony forming units, CFU-GM were cultured according to published procedures (Fibbe et al., J. Immunol. 148:417, 1992). Briefly, bone marrow cells were cultured in microtiter
10 plates containing 10^4 cells/well in semi-solid medium in the presence of murine GM-CSF (1.25 ng/ml). Peripheral blood mononuclear cells and spleen cells were cultured in 3.5 cm dishes containing 5×10^5 cells/ml and 10^6 cells/ml, respectively. Cells were cultured in a fully humidified
15 atmosphere at 37°C containing 5% CO₂. After 6 days of culture the number of colonies (defined as aggregates of >20 cells) were scored using an inverted microscope. The CFU-mix assay was performed in an identical fashion in 3.5 cm dishes in the presence of a combination of 1.25 ng/ml
20 recombinant murine GM-CSF, 2 U/ml recombinant human EPO, 25 ng/ml recombinant murine IL-3, 5% transferrin, 5% bovine serum albumin, 5% 10^{-3} b-mercaptoethanol, and 7.5% Iscove's modified Dulbecco's medium (IMDM). After 6 to 7 days of culture at 37°C in a fully humidified, 5% CO₂
25 atmosphere, the number of colony forming cells was scored using an inverted microscope. TPO treatment resulted in increased numbers of colony forming units (CFU) and BFU-Es in the bone marrow or spleen in comparison with saline-treated controls (Table).

Table			
<u>Donor Treatment</u>			
	<u>TPO</u>	<u>Saline</u>	
Femur			
Nucleated cells (x10 ⁶)	18.4 ± 4.7	19.9 ± 4.3	
CFU (x10 ³)	55.3 ± 12.5*	38.6 ± 5.2	
BFU-E (x10 ³)	24.0 ± 4.9	16.4 ± 2.3	
Spleen			
Nucleated cells (x10 ⁶)	71.8 ± 35.0	78.4 ± 42.5	
CFU (x10 ³)	27.3 ± 16.9	16.3 ± 11.4	
BFU-E (x10 ³)	10.2 ± 2.3	1.9 ± 0.7	
5	Results are expressed as absolute cell numbers (mean ± S.D., n=7) per organ (femur or spleen). CFU represents the total number of colonies cultured in the CFU-mix assay. *p<0.05.		

10 Lethally-irradiated recipient animals were
transplanted with 10⁵ bone marrow cells from donors that
had been treated with TPO at a dose of 20 kU/day i.p. for
five consecutive days, or from saline-treated control
donors. Blood samples were taken after transplantation
15 from individual recipients every 3 days by tail vein
bleeding. No difference in visible bleeding tendency was
observed between recipients of TPO-modified or unmodified
bone marrow cells.

20 Cell counts were analyzed using the student's T
tests. In the MANOVA analysis, groups were compared with
respect to their course over time. The analysis was
performed on the log values of the data. Values of <0.05
were considered statistically significant. Curves were
compared using the MANOVA test. Results showed that the
reconstitution of platelets in recipients of TPO-treated
25 marrow was significantly altered in comparison to control
animals transplanted with an equal number of bone marrow
cells from saline-treated control donors (Fig. 1). In
addition, platelet nadir counts were higher in animals

receiving TPO-treated marrow than those receiving control marrow (88×10^9 vs. 30×10^9 at day 12 after transplantation, mean of 20 mice). As shown in Fig. 1, post-transplant treatment with 20 kU/day TPO i.p. on days 1-5 did not result in a further acceleration of platelet reconstitution in mice that received marrow from TPO-treated donors.

In addition to an accelerated reconstitution of platelets, recipients of TPO-modified bone marrow cells also exhibited accelerated reconstitution of erythrocytes (Fig. 2). The erythrocyte nadir counts were also significantly higher in these animals than in controls transplanted with an equal number of unmodified bone marrow cells. Experiments were performed to further substantiate that this effect was due to a direct activity of TPO on erythropoiesis and not related to differences in platelet counts and bleeding tendency. In this experiment recipient animals were not bled until 12 days after transplantation, at which time the recipient mice were sacrificed, and the numbers of bone marrow and blood-derived progenitor cells were assessed. Recipients of TPO-modified bone marrow cells had a higher number of BFU-E colonies/femur (770 ± 386 vs 422 ± 320 , mean \pm SD, $n=5$) and higher reticulocytes in the blood (44% vs. 8%, mean of 5 mice) than controls transplanted with an equal number of unmodified bone marrow cells, although these differences did not reach statistical significance. Post-transplant treatment with TPO did not result in further acceleration of erythrocyte reconstitution at the doses tested.

Example 2

A second experiment was carried out to compare platelet counts in lethally irradiated mice receiving marrow from TPO-treated or non-treated donors, and to determine the effect of post-transplantation TPO treatment of the recipient animals.

B6D2 F1 mice were obtained from Taconic (Germantown, NY) and housed under specific pathogen-free conditions. The mice were housed five per cage and received acidified water and food *ad libitum*. Forty
5 female mice were used as recipients, and five male mice were used as donors.

Recombinant human TPO was prepared using transfected BHK 570 cells. The major molecular species was a 70 kD band. The preparation had a specific activity
10 of 5641 U/lg. The protein was made up in 29 mM potassium phosphate buffer, pH 6.0, containing 0.05% polysorbate 80 and 0.13 M NaCl and stored frozen in 20 kU aliquots. TPO and vehicle solutions were thawed directly before use and were injected into mice once daily, subcutaneously.

Two donor mice were each treated with 20 kU of
15 TPO per day for four days, then sacrificed by cervical dislocation on the fifth day. Control donors were treated with vehicle only. Femora were taken out aseptically, and bone marrow was flushed out with Ham's F12 (Fred
20 Hutchinson Cancer Research Center, Seattle, WA) containing 2% fetal bovine serum by inserting a 25 g. needle connected to a syringe. The cell suspension was flushed twice through an 18 g. needle, a 20 g. needle, and a 22 g. needle to produce a single-cell suspension. Nucleated
25 cells were counted in a hemocytometer.

On day -2, recipient mice were exposed to 1200 cGy total body irradiation from a ^{137}Cs source (Gammacell
40 Irradiator, Atomic Energy of Canada Radiochemical Company, Kanata, Canada). Bone marrow transplants were
30 performed two to four hours after irradiation. Twenty mice received bone marrow (1×10^5 cells) from TPO-treated donors, and twenty mice received 1×10^5 cells from vehicle-treated donors. Recipients were treated with TPO (20 kU/day) beginning on day 1 (2 days after transplantation)
35 and continuing for 14 days.

Mice were bled from the retroorbital sinus under ether anesthesia. Fifty 11 blood samples were collected

in heparinized micropipettes (VWR Scientific, Seattle, WA) and dripped into microtainer tubes with EDTA (Becton Dickinson, San Jose, CA). Blood was also dripped onto glass slides, and smears were prepared. Blood was
5 analyzed in a Cell Dyn 3500 hematology analyzer (Abbott, Santa Clara, CA). Hematocrit, RBC counts, WBC counts and platelet counts were determined.

In mice receiving marrow from control donors, platelet counts dropped on day 8 to low levels (below 6%
10 of normal) and started to recover in TPO-treated and control animals on day 12 (Fig. 3). There was no difference between the two groups in platelet recovery. However, in the vehicle-treated controls only 3 of 10 animals survived, whereas in the TPO-treated group 7 of 9
15 animals survived. Death was related to hemorrhage. Standard deviations were large within the TPO-treated group because some animals with very low platelet counts were able to survive.

Mice receiving marrow from TPO-treated donors
20 also had platelet numbers that were below 6% of normal on day 8. Animals that were treated with TPO for 14 days had, in general, a faster recovery in platelet counts. Eight of nine TPO-treated animals survived, whereas only four of nine vehicle-treated mice survived. RBCs
25 recovered faster in mice that received TPO-pretreated bone marrow and were treated with TPO compared to controls. There was no influence of TPO treatment on white blood cell recovery.

30 From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the
35 invention is not limited except as by the appended claims.

SEQUENCE LISTING

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 - (v) COMPUTER READABLE FORM:
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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1062 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1..1059

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ATG GAG CTG ACT GAA TTG CTC CTC GTG GTC ATG CTT CTC CTA ACT
GCA          48
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr
Ala
  1              5              10              15

AGG CTA ACG CTG TCC AGC CCG GCT CCT CCT GCT TGT GAC CTC CGA
GTC          96
Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg
Val
      20              25              30

CTC AGT AAA CTG CTT CGT GAC TCC CAT GTC CTT CAC AGC AGA CTG
AGC          144
Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu
Ser
      35              40              45

CAG TGC CCA GAG GTT CAC CCT TTG CCT ACA CCT GTC CTG CTG CCT
GCT          192
Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro
Ala
      50              55              60

GTG GAC TTT AGC TTG GGA GAA TGG AAA ACC CAG ATG GAG GAG ACC
AAG          240
Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr
Lys
      65              70              75
      80

GCA CAG GAC ATT CTG GGA GCA GTG ACC CTT CTG CTG GAG GGA GTG
ATG          288
Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val
Met
      85              90              95

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GCA GCA CGG GGA CAA CTG GGA CCC ACT TGC CTC TCA TCC CTC CTG
 GGG 336
 Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu
 Gly
 100 105 110

CAG CTT TCT GGA CAG GTC CGT CTC CTC CTT GGG GCC CTG CAG AGC
 CTC 384
 Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser
 Leu
 115 120 125

CTT GGA ACC CAG CTT CCT CCA CAG GGC AGG ACC ACA GCT CAC AAG
 GAT 432
 Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys
 Asp
 130 135 140

CCC AAT GCC ATC TTC CTG AGC TTC CAA CAC CTG CTC CGA GGA AAG
 GTG 480
 Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys
 Val
 145 150 155
 160

CGT TTC CTG ATG CTT GTA GGA GGG TCC ACC CTC TGC GTC AGG CGG
 GCC 528
 Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Arg
 Ala
 165 170 175

CCA CCC ACC ACA GCT GTC CCC AGC AGA ACC TCT CTA GTC CTC ACA
 CTG 576
 Pro Pro Thr Thr Ala Val Pro Ser Arg Thr Ser Leu Val Leu Thr
 Leu
 180 185 190

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 ACT 624
 Asn Glu Leu Pro Asn Arg Thr Ser Gly Leu Leu Glu Thr Asn Phe
 Thr
 195 200 205

GCC TCA GCC AGA ACT ACT GGC TCT GGG CTT CTG AAG TGG CAG CAG
 GGA 672
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 Gly
 210 215 220

TTC AGA GCC AAG ATT CCT GGT CTG CTG AAC CAA ACC TCC AGG TCC
 CTG 720

Phe Arg Ala Lys Ile Pro Gly Leu Leu Asn Gln Thr Ser Arg Ser
 Leu
 225 230 235
 240

GAC CAA ATC CCC GGA TAC CTG AAC AGG ATA CAC GAA CTC TTG AAT
 GGA 768
 Asp Gln Ile Pro Gly Tyr Leu Asn Arg Ile His Glu Leu Leu Asn
 Gly
 245 250 255

ACT CGT GGA CTC TTT CCT GGA CCC TCA CGC AGG ACC CTA GGA GCC
 CCG 816
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 Pro
 260 265 270

GAC ATT TCC TCA GGA ACA TCA GAC ACA GGC TCC CTG CCA CCC AAC
 CTC 864
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 Leu
 275 280 285

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 Tyr
 290 295 300

ACG CTC TTC CCT CTT CCA CCC ACC TTG CCC ACC CCT GTG GTC CAG
 CTC 960
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 Leu
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 320

CAC CCC CTG CTT CCT GAC CCT TCT GCT CCA ACG CCC ACC CCT ACC
 AGC 1008
 His Pro Leu Leu Pro Asp Pro Ser Ala Pro Thr Pro Thr Pro Thr
 Ser
 325 330 335

CCT CTT CTA AAC ACA TCC TAC ACC CAC TCC CAG AAT CTG TCT CAG
 GAA 1056
 Pro Leu Leu Asn Thr Ser Tyr Thr His Ser Gln Asn Leu Ser Gln
 Glu
 340 345 350

GGG TAA
 1062
 Gly

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 353 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Glu	Leu	Thr	Glu	Leu	Leu	Leu	Val	Val	Met	Leu	Leu	Leu	Thr
Ala														
1				5					10					15
Arg	Leu	Thr	Leu	Ser	Ser	Pro	Ala	Pro	Pro	Ala	Cys	Asp	Leu	Arg
Val														
				20				25					30	
Leu	Ser	Lys	Leu	Leu	Arg	Asp	Ser	His	Val	Leu	His	Ser	Arg	Leu
Ser														
				35				40					45	
Gln	Cys	Pro	Glu	Val	His	Pro	Leu	Pro	Thr	Pro	Val	Leu	Leu	Pro
Ala														
				50				55					60	
Val	Asp	Phe	Ser	Leu	Gly	Glu	Trp	Lys	Thr	Gln	Met	Glu	Glu	Thr
Lys														
65							70						75	
80														
Ala	Gln	Asp	Ile	Leu	Gly	Ala	Val	Thr	Leu	Leu	Leu	Glu	Gly	Val
Met														
							85				90			95
Ala	Ala	Arg	Gly	Gln	Leu	Gly	Pro	Thr	Cys	Leu	Ser	Ser	Leu	Leu
Gly														
							100				105			110
Gln	Leu	Ser	Gly	Gln	Val	Arg	Leu	Leu	Leu	Gly	Ala	Leu	Gln	Ser
Leu														
							115				120			125
Leu	Gly	Thr	Gln	Leu	Pro	Pro	Gln	Gly	Arg	Thr	Thr	Ala	His	Lys
Asp														
							130				135			140

Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys
 Val
 145 150 155
 160

Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Arg
 Ala
 165 170 175

Pro Pro Thr Thr Ala Val Pro Ser Arg Thr Ser Leu Val Leu Thr
 Leu
 180 185 190

Asn Glu Leu Pro Asn Arg Thr Ser Gly Leu Leu Glu Thr Asn Phe
 Thr
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Ala Ser Ala Arg Thr Thr Gly Ser Gly Leu Leu Lys Trp Gln Gln
 Gly
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Phe Arg Ala Lys Ile Pro Gly Leu Leu Asn Gln Thr Ser Arg Ser
 Leu
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 Gly
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 Pro
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 Leu
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Gln Pro Gly Tyr Ser Pro Ser Pro Thr His Pro Pro Thr Gly Gln
 Tyr
 290 295 300

Thr Leu Phe Pro Leu Pro Pro Thr Leu Pro Thr Pro Val Val Gln
 Leu
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 320

His Pro Leu Leu Pro Asp Pro Ser Ala Pro Thr Pro Thr Pro Thr
 Ser
 325 330 335

Pro Leu Leu Asn Thr Ser Tyr Thr His Ser Gln Asn Leu Ser Gln
Glu

340

345

350

Gly

Claims

1. A method for increasing hematopoietic cells in a recipient patient in need of such increase comprising:

administering to a donor an amount of thrombopoietin (TPO) sufficient to stimulate proliferation of cells of the myeloid lineage in the donor;

collecting cells from the donor, wherein the cells are bone marrow cells or peripheral blood stem cells;

administering the bone marrow cells or peripheral blood stem cells to a recipient patient.

2. A method according to claim 1 wherein the recipient patient has been treated with chemotherapy or radiation therapy.

3. A method according to claim 1 wherein the donor and the recipient patient are the same individual.

4. A method according to claim 3 wherein the recipient patient is treated with chemotherapy or radiation between the collecting and second administering steps.

5. A method according to claim 1 wherein the cells are bone marrow cells.

6. A method according to claim 1 wherein the cells are peripheral blood stem cells.

7. A method according to claim 1 further comprising administering to the recipient patient, after or concurrently with administering the bone marrow cells or peripheral blood stem cells, an amount of

thrombopoietin sufficient to enhance platelet recovery or erythrocyte recovery.

8. A method according to claim 1 wherein the TPO is human TPO.

9. A method of preparing cells for transplantation comprising:

administering to a donor an amount of thrombopoietin (TPO) sufficient to stimulate proliferation of cells of the myeloid lineage in the donor;

collecting cells from the donor, wherein the cells are bone marrow cells or peripheral blood stem cells.

10. A method according to claim 9 wherein the TPO is human TPO.

11. A method according to claim 9 wherein the cells are bone marrow cells.

12. A method according to claim 9 wherein the cells are peripheral blood stem cells.

13. A method of stimulating platelet or erythrocyte recovery in a patient receiving chemotherapy or radiation therapy comprising:

administering to the patient an amount of TPO sufficient to stimulate proliferation of cells of the myeloid lineage in the patient;

collecting bone marrow cells or peripheral blood stem cells from the patient prior to chemotherapy or radiation therapy; and

returning the collected cells to the patient subsequent to chemotherapy or radiation therapy.

14. A method according to claim 13 further comprising administering to the patient, after or concurrently with returning the collected cells, an amount of thrombopoietin sufficient to enhance platelet recovery or erythrocyte recovery.

15. A method according to claim 13 wherein the TPO is human TPO.

16. A method according to claim 13 wherein the cells are bone marrow cells.

17. A method according to claim 13 wherein the cells are peripheral blood stem cells.

FIG. 1

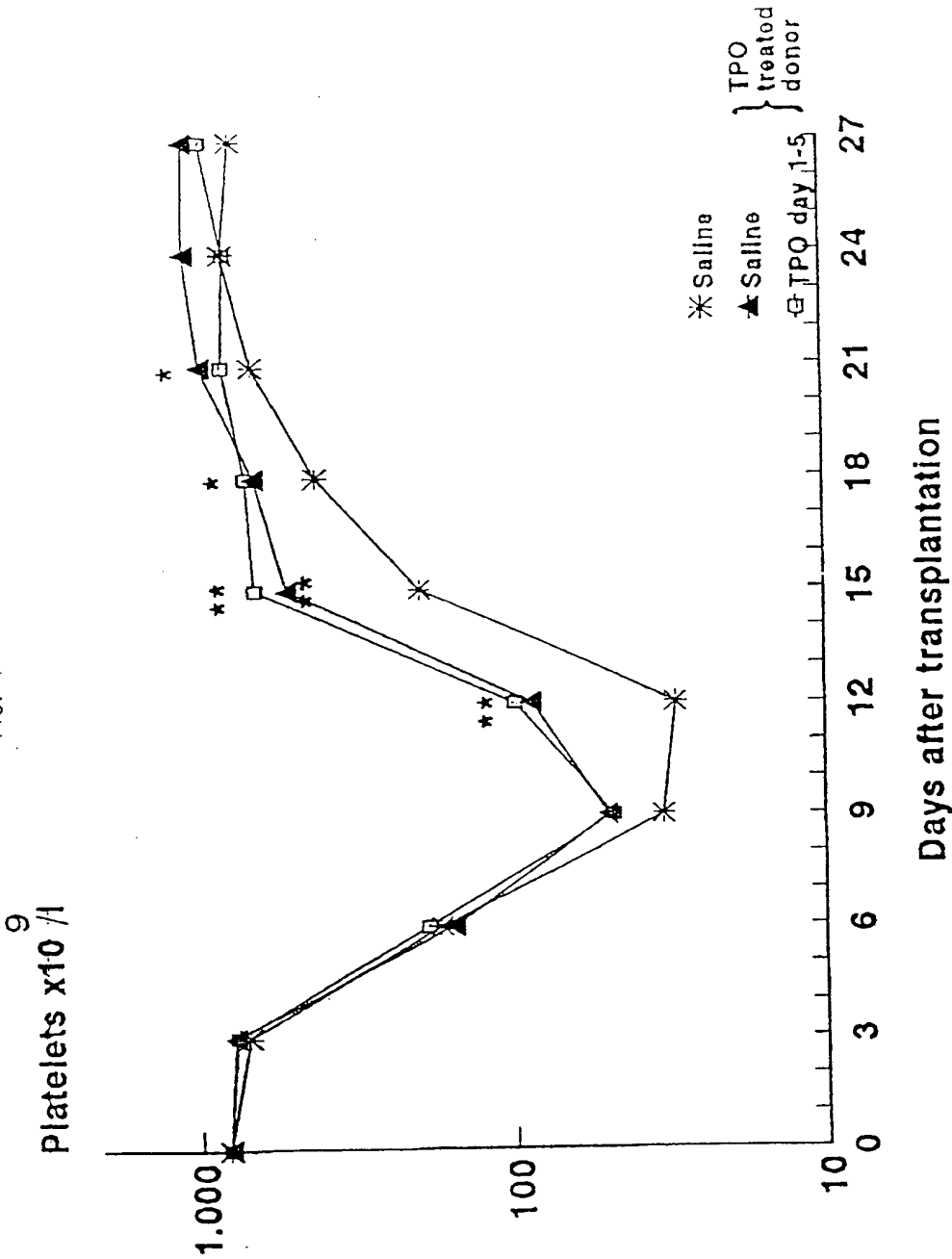


FIG. 2

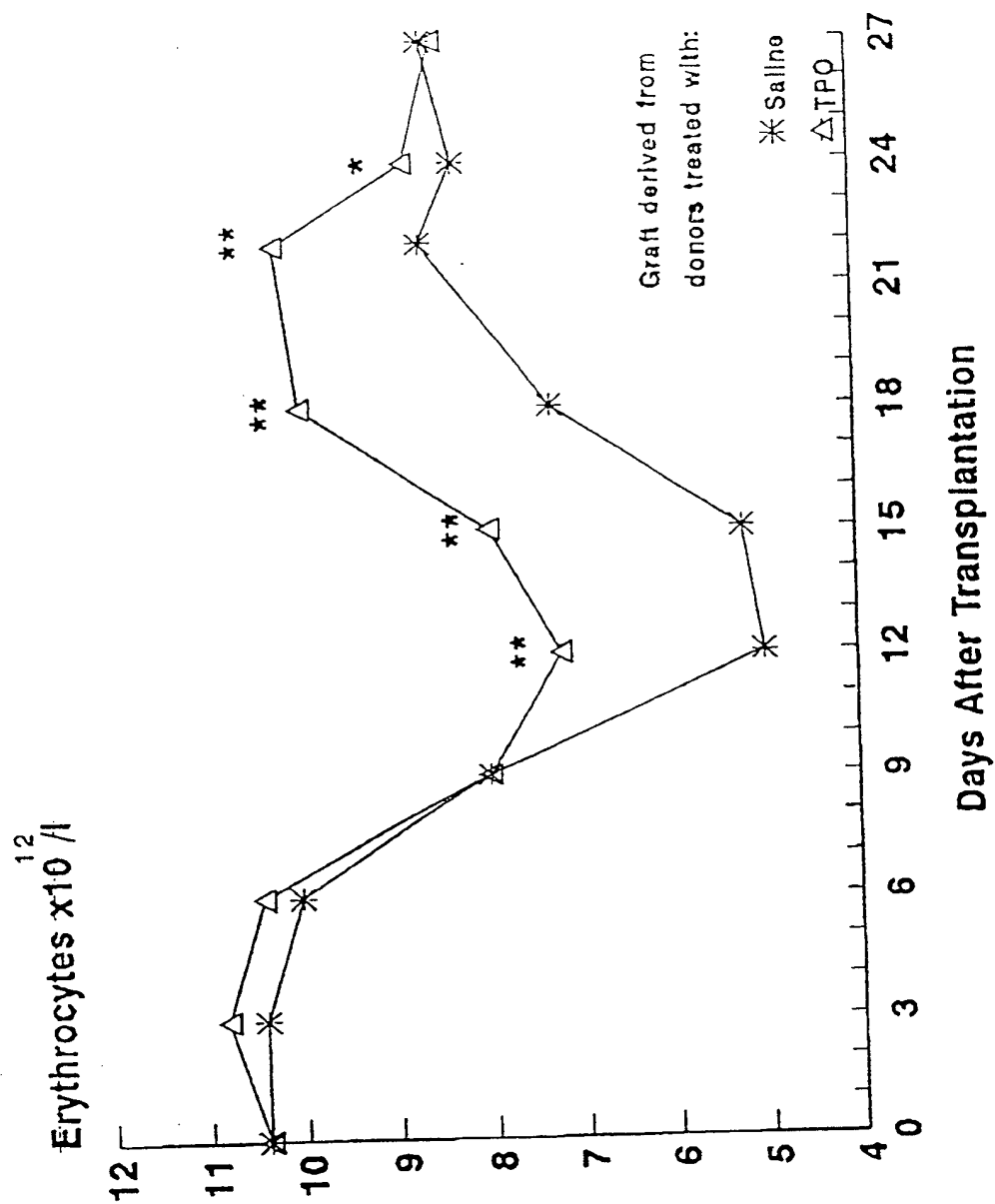
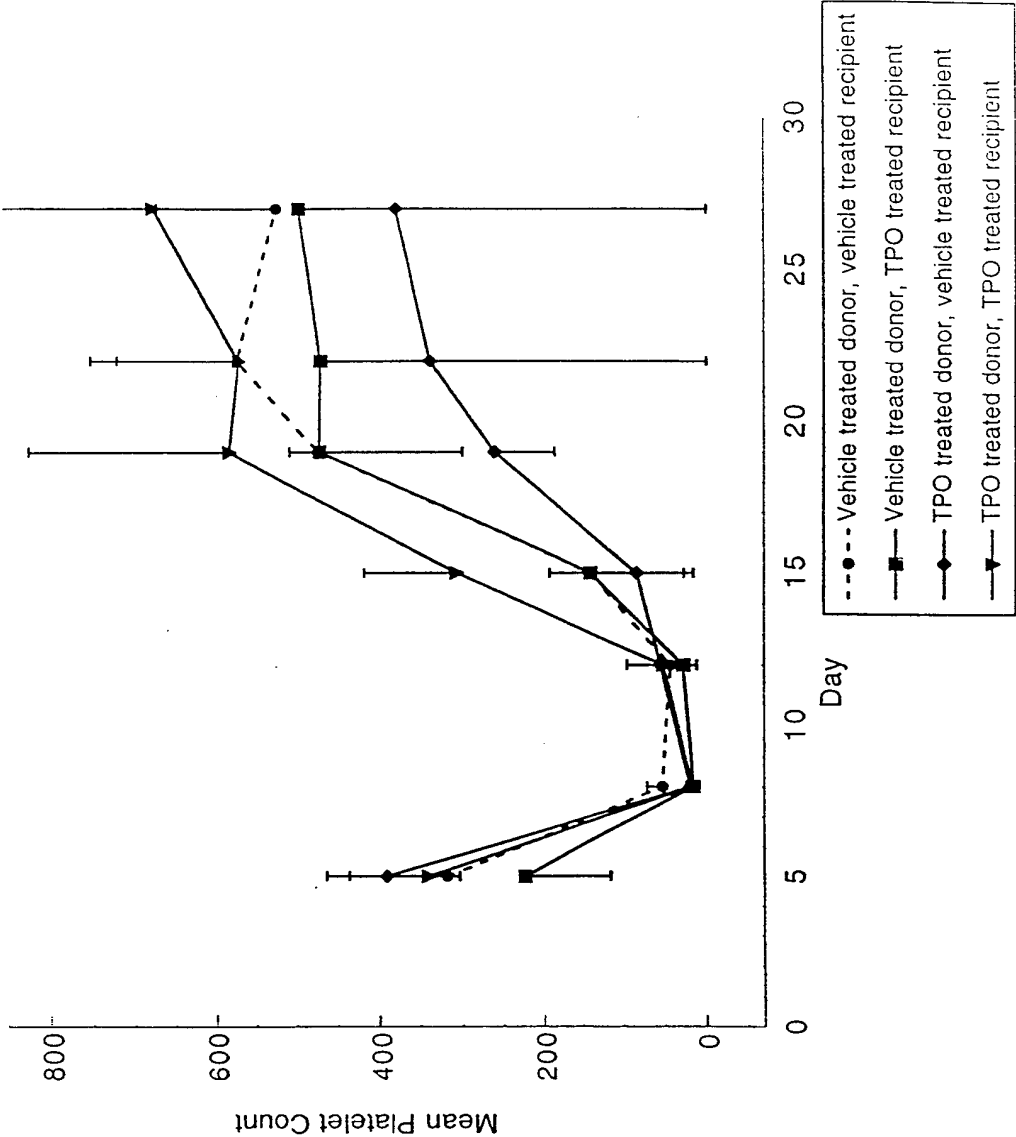


FIG. 3



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/07880

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/19 A61K35/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 369, 1994, LONDON GB, pages 519-520, XP002013270 DONALD METCALF: "Thrombopoietin-at last" see page 520, columns 2-3 ---	1-17
A	LANCET THE, vol. 339, 1992, LONDON GB, pages 640-644, XP002013271 SHERIDAN, W.P. ET AL: "Effect of peripheral-blood progenitor cells mobilised by filgrastim (G-CSF) on platelet recovery after high-dose chemotherapy" see the whole document --- -/-	1-17

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

13 September 1996

Date of mailing of the international search report

24.09.1996

Name and mailing address of the ISA

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Fax: (+ 31-70) 340-3016

Authorized officer

Fernandez y Branas, F

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PC1/US 96/07880

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	<p>BLOOD, vol. 86, no. 9, 1 November 1995, pages 3308-3313, XP000602034 FIBBE W.E. ET AL: "Accelerated reconstitution of platelets and erythrocytes after syngenic transplantation of bone marrow cells derived from thrombopoietin pretreated donor mice" see the whole document ---</p>	1-17
A	<p>BLOOD, vol. 84, no. 10, 1994, page 242a XP002013272 SPRUGEL K.H. ET AL: "Recombinant thrombopoietin stimulates rapid platelet recovery in thrombocytopenic mice" see abstract 952 ---</p>	1-17
A	<p>NATURE, vol. 369, 1994, LONDON GB, pages 533-538, XP002013273 FREDERIC J. DE SAUVAGE: "Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-MPL ligand" see the whole document ---</p>	1-17
A	<p>STEM CELLS, vol. 12, no. 1, 1994, pages 91-97, XP002013274 KAUSHANSKY K. : "The mpl ligand: Molecular and cellular biology of the critical regulator of megakaryocyte development" see the whole document ---</p>	1-17
A	<p>NATURE, vol. 369, 1994, LONDON GB, pages 568-571, XP002013275 KAUSHANSKY K. ET AL: "Promotion of megakaryocyte progenitor expansion and differentiation by the c-MPL ligand thrombopoietin" see the whole document -----</p>	1-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/07880

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-8, 13-17
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.